Fall 2018

Varying Water Stress in Mimulus ringens

Sara Stiles
ses132@zips.uakron.edu

Varying Water Stress in *Mimulus ringens*

By: Stiles S. and Mitchell R.

11/20/18

Abstract

A wetland plant's ability to tolerate flooding is important to determining where that plant can grow. Previous studies have shown the optimal flood tolerance of *Mimulus ringens* is between -2cm and -6cm (Fraser & Karnezis, 2005). This experiment expands on these previous experiments by testing variation in water levels instead of maintaining the water levels at one height throughout the experiment. The hypothesis of the experiment is that *Mimulus ringens* that have variation in water levels will show signs of better growth than plants with a constant water level. Contrary to predictions, the results showed the final height, number of total flowers produced, and above ground mass of varied water plants were significantly smaller than plants experiencing constant water levels. This is the opposite of field observations in which plants in areas of wetlands that experienced variation in their water levels had better overall growth in terms of height. Which suggests that the method of water level variation in this experiment maybe different than the variation that occurred in the previously observed wetlands.

Introduction

Wetlands are important as they are natural filters for water (EPA, 2006). They provide many benefits such as cleaning water sources, controlling floods, and providing habitats for fish and other plants and animals (EPA, 2006). In wetlands the level of water is greatly important in determining what types of plants can and cannot grow there (Keddy, 2010). There are many different types of wetlands that vary in their water levels and sources, including swamps, marshes, bogs, fens and two sub-classes of wet meadows and shallow water (Keddy, 2010). Wetlands that do not have consistent water levels have more diversity, as these conditions allow for many different types of plants that require different amounts of flooding to grow (Keddy, 2010). A plants' flood tolerance is the "primary constraint" in wetland plants, as soils that are inundated with water are hypoxic (Keddy, 2010). Hypoxic soils have low oxygen concentrations which put added stress on the roots of a plant (Parent, Capelli, Berger, Crevècoeur, & Dat, 2008). *Mimulus ringens* for example cannot tolerate a water level above the surface of the soil (Fraser & Karnezis, 2005). In a 2008 study by Parent et al., an experimental group of plants were exposed to variable water levels showed that varying water can change this hypoxic condition in the soils as the control group will have more hypoxic soil than the experimental group (Parent, Capelli, Berger, Crevècoeur, & Dat, 2008).

Water stressing of wetland plants, including *Mimulus ringens,* has been researched greatly. Previous experiments have found that a constant high-water level that is around 2 cm below the soil surface leads to the most successful growth in a greenhouse environment (O'Halloran & Carr, 2010). However, the effects of varied water stress have not been thoroughly examined. In the field we observed that monkey flower plants in areas of the Bath Nature Preserve that receive greatly varying amounts of water appeared to be larger than plants in areas that received a consistent amount of water (Professor R. Mitchell, personal communication, November 15, 2017). This observation led me to investigate the effects of varied water levels on *Mimulus ringens*. Fraser and Karnezis showed that *Mimulus ringens* have the best growth rate when water levels are below surface level, between 2 and 6 cm below the surface (2005). Like most studies Fraser and Karnezis stressed plants by maintaining a constant level of water for each individual plant above or below the surface of the soil (2005). However, the effects of varying water level on the same monkey flower plant, as opposed to just a constant water level, has yet not been investigated.

The purpose of this project was to determine if varying the amount of water level improves *Mimulus ringens* growth. *Mimulus ringens* is a type of wetland plant that is commonly referred to as Allegheny monkeyflower (USDA, 2017). This research provides information about the growth of monkey flowers that could be used during future research and in wetland restoration. In this experiment the water level for the first test group will be set at a maximum of -2 cm for 2 days and then lowered to no water at all, alternating the water level every two days throughout. The second group is the control group, in which plants will receive a constant water level of 2 cm below the surface of the soil. This control group will constantly have water stress as they will have a constantly high level of water. From this project further information about wetland plants undergoing water stresses was gained. Valuable information that could aid in the growth of *Mimulus ringens* will be gained. This project could help researchers grow plants more quickly and with lower mortality rates.

Methods and Materials

Greenhouse

The greenhouse at the University of Akron was the location of this experiment. This greenhouse is not able to maintain constant temperatures during extremely cold periods, and therefore had to be heated using a portable heater in the winter month of the experiment to provide as close to the optimal greenhouse growing temperature between 70-76ºF (Professor J. Karron, UW Milwaukee, personal communication, December 18, 2017). Heatwaves during the summer months were also an issue for the varied water plants, as two times during the experiment they experienced partial wilting on days with high outside temperatures. The first heating event occurred on May 27th and the second occurred on June 16th. The lighting plants received was the natural photoperiod as the sunlight entering the green house during daylight hours provided light.

To Start Seeds

To begin growing the plants three 17x2x8cm seed propagators with no holes in the bottom were filled with fine soil (Sungrow Horticulture Professional Growing Mix) on December 18th. Water was then added until the soil was extremely moist, but did not have any pools of water on the surface. About 50

Mimulus ringens seeds collected in October from Windhover Bog at the Bath Nature Preserve, were added to each propagator making sure to spread them out evenly. A clear plastic lid to maintain a humid atmosphere was placed on the propagator and were watered as needed to maintain moist soil. Seedlings emerged after 5 days, reaching the two-leaf stage about 12 days after planting.

Transplanting Seeds into First Set-up

After three weeks I transplanted the young seedlings (~3mm tall) into individual cells for the initial experimental set-up. To do this I filled 15 13.5x18x8cm seedling starter trays that have 6 cells each with fine potting soil (Sungrow Horticulture Professional Growing Mix). I placed the seedling starter trays individually into 17x23x8cm propagators with holes, for ease of moving plants around. I then placed three propagators into a 26.5x53x8cm large tray and filled it until the water level was 2 cm below the surface of the soil (Figure 1). One of the remaining seedling starter trays containing plants was placed into its own large tray and the other two were placed into their own large tray. I then transplanted individual plants into their own individual cells. I chose which plants to transplant randomly and not based on size. In total there were 42 varied group plants and 43 constant group plants for a total of 85 plants.

Water variation

Half of the plants received a varied water treatment and the other half of plants received constant water. Every 2 days, all water was removed from 3 of the large trays, this group of 42 plants are the experimental group for the water stress test. Emptying and refilling of varying water level plants was repeated every 2 days throughout the experiment. For the other 43 control plants water level was refilled to ensure it remained at 2 cm below the surface of the soil. Then 2 days later both the varied water and constant water trays were refilled to 2 cm of water below the surface. Also, every two days all of the trays in both groups were moved randomly to new positions to prevent the positioning of the plants from causing any major differences in the results of the experiment. There were periods during the hot summer months when even the constant water treatment tray would dry down quite far. So, water level of these plants also had a variation of their own as there were only refilled every 2 days. The two-day cycle was chosen based on pre-trials, where plants left without water for a period longer than 2-3 days would dry out considerably. This water variation treatment was done for a total of 274 days, from mid-December to mid-September.

Transplanting into Second Set-up

At the end of month 3, when plants reached a size too large for the cells, plants were transplanted into 4-inch square pots and slow release fertilizer was added. The fertilizer used in this experiment was the recommended .1296 ounces per pot of Scotts Osmocote 14-14-14 slow release fertilizer. In this new set-up 9 pots from the same treatment group were placed into a large tray and filled with water until -2 cm below the surface of the soil. This step was then repeated until there was a total of 10 large trays, 5 large trays for the experimental varied group and 5 large trays for the constant -2 cm of water group (Figure 2). Varying the water levels of the experimental varied water group was continued in the same manner as in the first 3 months of the experiment.

Data Collection and Statistics

Plants at the end of the experiment were similar in size and produced a similar number of flowers to plants that are grown naturally in wetlands. The plants also started the normal fall senescence seen in naturally grown *Mimulus ringens*. Collection of height data and the number of plants present above ground (with green leaves) was recorded each month throughout the experiment. Height was measured from the surface of the soil to the tallest point of the plant as it was sitting. Data collected on September 17th, the final day of the experiment, includes height, shoot mass above ground, root mass below ground, and the total number of flowers produced. Roots were cleaned by soaking pots in containers of water to loosen soil and then spraying soil off completely using a garden hose until no soil remained. For mass measurements plants were dried in a drying oven at 65°C for 3 days. The statistical software JMP was for analysis of the data gathered. The first type of statistical test was ANOVA for final height, mass above ground, mass below ground, and total flowers produced data. The second type of statistical test performed was a Chi-Square test for the final number of plants alive. Statistical tests were also done to ensure there was no significant variation within experimental groups of plants that were group in different large trays.

Results

Throughout the experiment plants with varied water levels performed worse than plants with a constant water level. Significant results include height, flowers produced, and above ground mass which were all greater in constant water plants than in varied water plants.

Height

At experiment end (after 274 days) height of varied water treatment plants was significantly smaller than those in the constant water treatment ($F_{1,64} = 68.9$, $p < 0.0001$). A bar graph of these averages is shown in Figure 3. Plant heights of constant treatment plants were larger throughout the experiment (Figure 4).

Total Flowers Produced

The total number of flowers produced by varied plants was significantly smaller than that of constant water plants ($F_{1,64} = 24.6$, $p < 0.0001$). For analysis the data was transformed to the logarithmic values to obtain a more normal distribution, but the untransformed means are shown in Figure 5 for ease of interpretation.

Mass above ground

Above ground mass of varied water treatment plants was significantly smaller than that of constant water treatment plants ($F_{1,64} = 8.3$, $p < 0.0001$) (Figure 6).

Mass below ground

Below ground mass of varied water treatment plants was insignificantly smaller than constant water treatment plants ($F_{1,64} = 0.005$, $p< 0.0001$) (Figure 7). The mean below ground mass of varied plants was only 0.1 grams smaller than constant plants out of a mean of 10 grams which, while statistically significant, is biologically insignificant.

Final Number of Plants Alive

There was no difference between the treatments in final number of plants alive at harvest, ($X^2= 0.24$, $p = .62$). There was a resurrection event that took place during month 6 of the experiment (June). During this resurrection 11 plants that were not present above ground at the end of month 5 returned by the end of month 6 (Figure 8). Overall survival of constant water plants was 31/43= 72%, while the overall survival of varied water plants was 35/42= 83%.

Discussion

The hypothesis that variation in water levels would improve growth in *Mimulus ringens* was shown to be incorrect. However, there was a significant difference in plant size and production of flowers between the constant water level and varied water level groups, in the other direction. Significant results gathered include final heights of plants, total number of flowers produced, and above ground mass, in all of which constant plants had significantly higher values. A possible explanation for plants with constant high-water level having better growth was seen in a wetland field experiment on trees which had a similar result, as trees with constant flooding had a greater weekly diameter growth than trees that experienced droughts and varied flooding (Keeland & Sharitz, 1997). This difference was possibly due to varied water trees having to constantly restructure their roots in order to adjust for constantly changing water levels while trees in a constant water level did not have to restructure their roots (Keeland & Sharitz, 1997). This shows plants with consistent water levels can grow more successfully than plants with varied water even with the soil experiencing more hypoxic conditions.

The finding that constant water plants had better growth contradicted the hypothesis (based on field observations) that varied water leads to improved growth. Other experiments done involving the effects of different constant water levels on *Mimulus ringens* have also seen a difference between greenhouse and field plants in terms of growth (O'Halloran & Carr, 2010). An experiment by O'Halloran and Carr for example found that a constant high-water level (-2cm below the surface) provided the best overall growth in a greenhouse but a medium constant water level provided the best growth in their field test (O'Halloran & Carr, 2010). The difference between my field observations and my experimental results could be due to many reasons. One reason for the difference could be the experimental setup being in a greenhouse instead of outdoors. Another difference is the temperatures at which that plants were grown. Plants were first transplanted in December when the greenhouse was cold and a heater was added to attempt to provide heat. Another important difference could be the levels at which water variation was set. Varied plants had all water removed for alternating 2-day periods. In the field variation in water level could have been less extreme.

However, I found no significant effect of varied level on below ground mass and final number of plant alive tendencies. The mass below ground was not significantly different between the two groups. This shows the root masses of the two groups are similar to one another in weight. The difference in survival of plants between the two groups was also insignificant. However, there was also a resurrection event that was observed during the experiment. At the end of month five (May) there were only 20 constant water plants present above ground, but by the end of the experiment there were 31 constant water plants present above ground (Figure 8). This indicates that the plants were not truly dead and new offshoots were able to grow back up after the originals had died. This could be explained by the fertilizer added when plants were transplanted into larger pots in month 4, the new soil added to fill the larger pot size, or the greenhouse temperature increasing due to outside temperature increase in later months of the experiment. This resurrection of plants could also be related to the two heating events in the summer as it caused the plants that experienced the varied water treatment to wilt. The first wilting event occurred a week after measurements were recorded in May, when the varied plants had only 20 plants alive, and the second occurred a week before measurements were recorded in June, when the plants had stared to grow above the soil surface again.

Moving forward, if this experiment were to be repeated in an attempt to achieve results closer to the field observations many changes would need to be made. The first change is adding more groups of variation to the experiment. As *Mimulus ringens* have been shown to have the best growth at water levels between 2 and 6 cm below the surface, the new groups added should fall in this range (Fraser & Karnezis, 2005). For example, groups that vary between -2cm and -4cm, -2 and -6cm, and so on. This would allow for more types of variation within the optimal water level range and less extreme variation than the complete removal of water done in this experiment. These conditions would be more similar to the observed field sites than the previous set-up. A change could also be made in the number of days between varying or adding water. Two days were chosen for this experimental set-up as plants without any water for any longer would begin to die. Longer intervals between water level manipulation could be possible if the varied groups do not have all water removed. Another change that would be made is starting with the larger pot set-up and adding the fertilizer at the start of the experiment instead of halfway through. This could possibly prevent plants that were no longer present above ground from having offshoots that grow back up in later months. This experimental set-up would provide a more thorough approach to determining the effects of varied water levels on *Mimulus ringens*.

Acknowledgments

I would like to thank Dr. Mitchell with his guidance and help throughout the process of this project.

Literature Cited

Environmental Protection Agency (EPA). (2018, January 19). *Why are wetlands important?* Retrieved from EPA.gov: https://www.epa.gov/wetlands/why-are-wetlands-important

Fraser, L. H. and J. P. Karnezis (2005). A comparative assessment of seedling survival and biomass accumulation for fourteen wetland plant species grown under minor water-depth differences. *Wetlands*, *25(3)*, 520-530. Retrieved from https://doi.org/10.1672/0277-5212(2005)025[0520:ACAOSS]2.0.CO;2

Keddy, P.A. (2010). *Wetland Ecology Principles and Conservation* (2nd ed.). Cambridge: Cambridge University Press.

Keeland, B.D. and R.R. Sharitz (1997). The effects of water-level fluctuations on weekly tree growth in a southeastern USA swamp. *American Journal of Botany 84(1)*, 131-139. Retrieved from https://onlinelibrary.wiley.com/doi/pdf/10.2307/2445890

O'Halloran, L. R. and D. E. Carr (2010). Phenotypic plasticity and inbreeding depression in Mimulus ringens (Phrymaceae). *Evolutionary Ecology Research 12(5)*, 617-632. Retrieved from http://www.evolutionary-ecology.com/abstracts/v12/2540.html

Parent, C., Capelli, N, Berger, A., Crevècoeur, M., & Dat, J. (2008). An Overview of Plant Responses to Soil Waterlogging. Plant Stress. *2(1)*, 20-27. Retrieved from https://www.researchgate.net/publication/278810941_An_Overview_of_Plant_Responses_to_Soil_Waterlogging

USDA. (2017). *Mimulus ringens L. Allegheny monkeyflower*. Retrieved from United States Department of Agriculture: https://plants.usda.gov/core/profile?symbol=miri

Figures

Figure 1. Photo of experimental set up. This photo shows the set-up of the experiment. Seedling starter trays with 6 cells placed within large trays. The first set-up consisted of six of these trays.

Figure 2. Photo of second stage of experimental set-up. 9 pots placed within a large tray. The second set-up consisted of 10 of these trays.

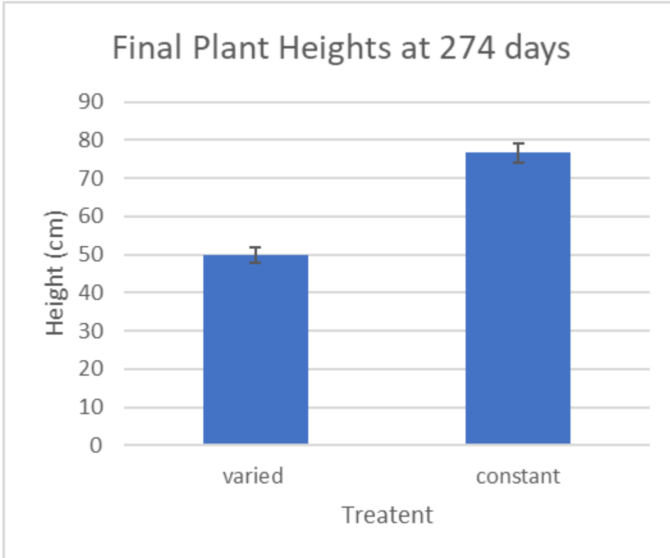

Figure 3. Average Final Height. A bar graph of the average final heights of each treatment with standard error bars ($F_{1,64} = 68.9$, $p<0.0001$) varied N=35/bar and constant N=31/bar.

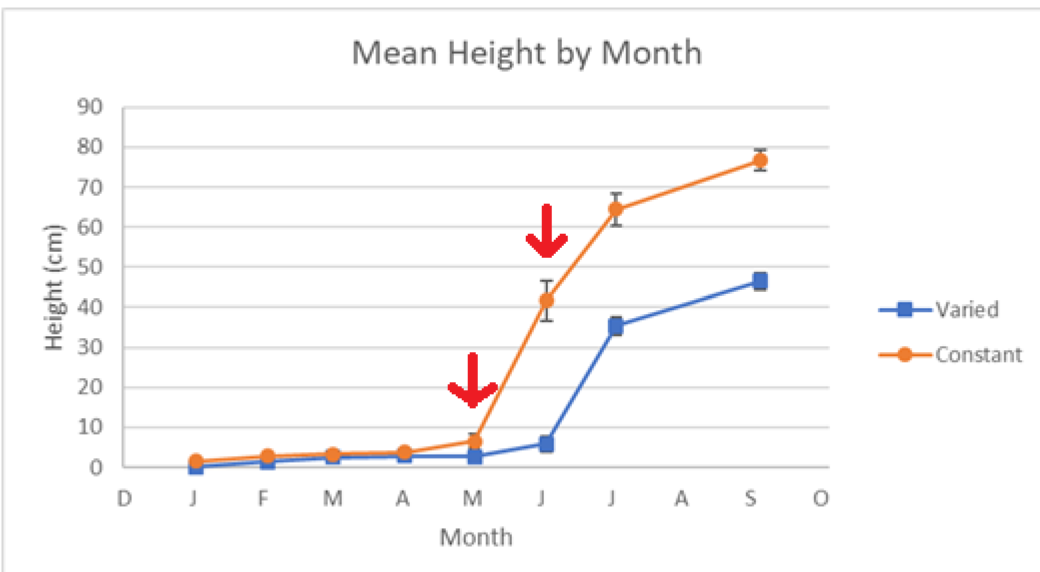

Figure 4. Average Height by Month. A scatter plot of the monthly average heights of plants in each group with standard error bars. Months in which the two heating and wilting events took place, May and June, are indicated by red arrows.

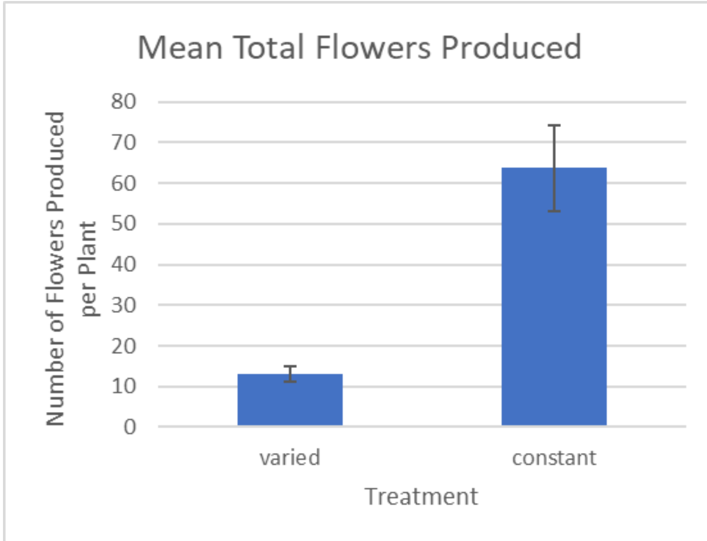

Figure 5. Average Total Flowers Produced. A bar graph of the average total mass above ground of each treatment with standard error bars ($F_{1,64}$ = 24.6, p<0.0001) varied N=35/bar and constant N=31/bar. ANOVA was performed on log transfer but untransformed means are shown in figure.

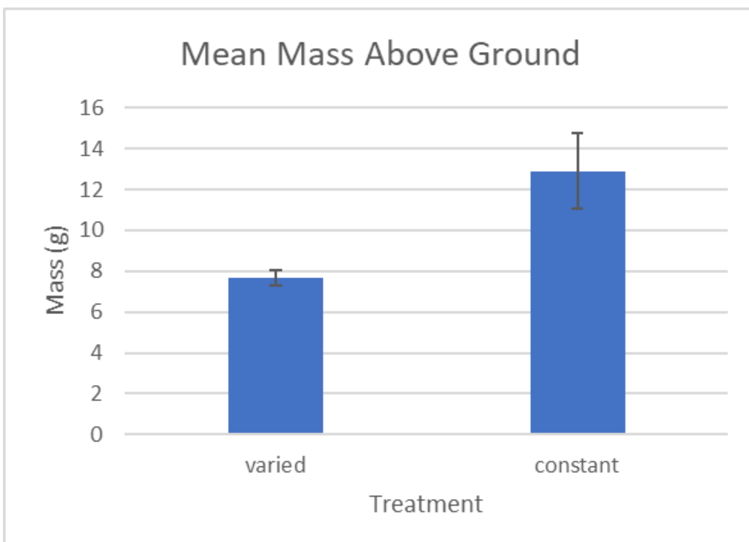

Figure 6. Average Mass Above Ground. A bar graph of the average mass above ground of each treatment with standard error bars ($F_{1,64}$ = 8.3, p<0.0001) varied N=35/bar and constant N=31/bar.

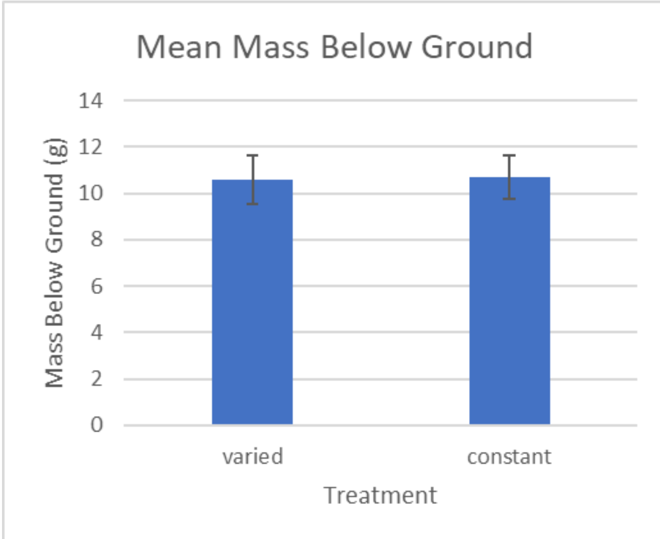

Figure 7. Average Mass Below Ground. A bar graph of the average mass below ground of each treatment with standard error bars ($F_{1,64} = 0.005$, $p<0.0001$) varied N=35/bar and constant N=31/bar.

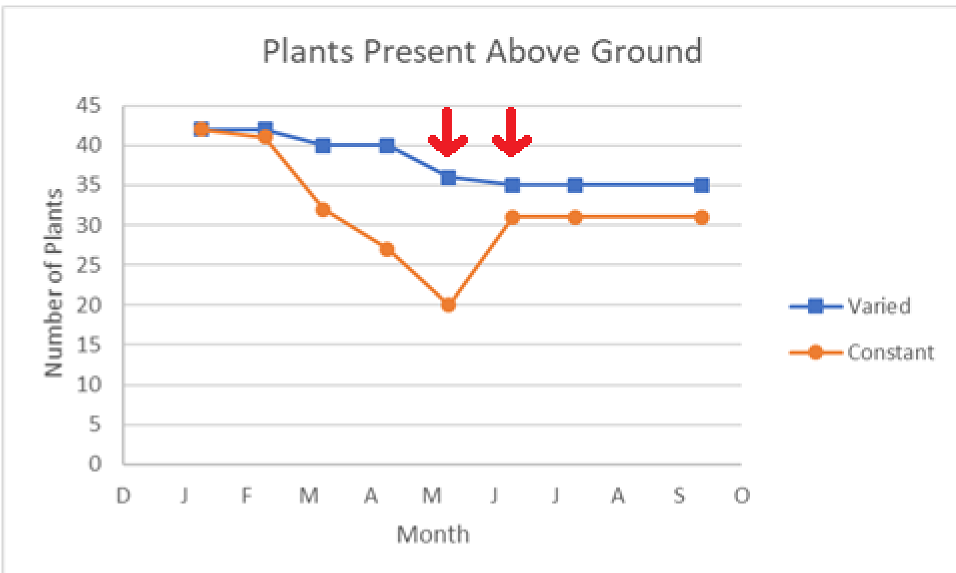

Figure 8. Plants Present Above Ground. A scatter plot of the total number of plants with measurable mass above ground in each month for each treatment including the final number of plants present (X2 = 0.24, p = .62). Months in which the two heating and wilting events took place, May and June, are indicated by red arrows.